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ANAEROBIC GROWTH OF MOLDS ISOLATED FROM FERMENTATION STARTERS USED FOR FOODS IN ASIAN COUNTRIES

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ABSTRACT

Ragi, murcha, look pang, bubod, chiu-chu, and Chinese yeast are used as starters for a number of fermentations based on rice and cassava in the Orient. The starter consists regularly of certain species of *Mucor, Rhizopus,* and *Amylomyces* and not of other molds, even though the production of starters is often made under unsanitary conditions. All 60 isolates from these starters from Indonesia, Philippines, Nepal, China, Taiwan, and Thailand grew under anaerobic conditions. The *Mucor* isolates belong in the section *Racemosus* and possess numerous chlamydospores. A survey of strains representative of 12 families of the Zygomycetes indicates that species of most genera will not grow under anaerobic conditions. The three genera in which some species grow under anaerobic conditions are *Mucor, Rhizopus,* and *Amylomyces* (Mucorales: Mucoraceae). *Amylomyces* is like *Rhizopus* in that growth is strictly filamentous under anaerobic conditions, further proving their close relationship. Heterothallic *Mucor* mating types of the species that grew under anaerobic conditions failed to produce zygospores when tested under anaerobic conditions. One homothallic species likewise failed to produce zygospores. In *Amylomyces,* chlamydospore production was almost completely suppressed under anaerobic conditions. None of the species tested formed sporangia in the absence of oxygen. When the starter cultures of *Mucor* were tested with 5% CO_2, 10% H_2, and 85% N_2 in an anaerobic system, growth was similar to that of cultures in an aerobic environment; however, the growth was greatly reduced when CO_2 was omitted from the anaerobic mixture (10% hydrogen, 90% nitrogen).

Key Words: anaerobic growth, zygospores, Zygomycetes, *Mucor, Rhizopus, Amylomyces,* fermentation starters.

In the production in Asia of various foods based on starchy commodities, often purposely mixed cultures consisting of molds, yeasts, and bacteria are used as starters. Starters invariably are used in nonaerated fermentations. The starter preparations go under a variety of names, such as murcha in North India and Nepal, ragi in Indonesia, look pang in Thailand, bubod in the Philippines, and Chinese yeast or chiu-chu in Taiwan and China (Hesseltine, **1983**). Surprisingly, these starters have few contaminating microorganisms. In our study of the kinds and numbers of species present, one is struck by the uniform occurrence of only certain species of *Mucor, Rhizopus,* and *Amylomyces,* to the exclusion of other fungi, even though the companies making and selling the starters produce them under unsanitary conditions, apparently with little or no knowledge of the microbiology involved. While examining the growth of some of the *Mucor* isolates on agar in unslanted tubes, we noticed much growth of the mycelium several centimeters below the surface. This indicated to us that the organisms were grow-

[1] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

ing better under reduced oxygen levels and prompted our examination of the anaerobic growth of molds from starter materials.

Anaerobic growth of fungi has been known for a long time, and the earlier literature has been reviewed thoroughly by Tabak and Cooke (**1968**). Hawker (**1966**) states that "While all, or nearly all, fungi have an absolute requirement for oxygen in the atmosphere, the actual concentration needed is usually rather low but may be higher for reproduction than for vegetative growth." According to this last author, the fact that Mucorales produce alcohol under low oxygen tensions indicates that they also possess the ability to metabolize anaerobically. Introduction of carbon dioxide into an anaerobic atmosphere produces spherical budding of yeast-like cells in *Mucor rouxii* (Calm.) Wehmer (Bartnicki-Garcia and Nickerson, **1959**, **1962**; Safe, **1973**). Bartnicki-Garcia and Nickerson studied several species of *Mucor* [*M. rouxii, M. subtilissimus* Oud., *M. racemosus* Fres., *M. mucedo* (Fr.) Fres., and *M. ramannianus* Moeller]. Under a CO_2 atmosphere most produced yeast-like cells except *M. ramannianus,* which did not grow and may not even belong in the genus *Mucor.* They also showed that five of the strains of *Rhizopus* grew under CO_2 but showed only filamentous growth. However, strains of *Mortierella, Syzygites* (*Sporodinia*), *Cunninghamella, Circinella, Phycomyces,* and *Zygorhynchus* failed to grow in a CO_2 atmosphere.

In recent years, the growth of *Mucor* has been studied in relation to metabolism of glucose (Inderlied and Sypherd, **1978**), lipid synthesis during morphogenesis (Ito *et al.,* **1982**), chitin synthesis (Domek and Borgia, **1981**), and nutritional requirements for growth and yeast-like development (Elmer and Nickerson, **1970**). Herber *et al.* (**1983**) reported that sterol production by cultures of *Mucor hiemalis* Wehmer differed qualitatively and quantitatively when grown under strict anaerobic conditions. However, there appear to have been no studies of the effect of anaerobic conditions on sexual reproduction in Mucorales or other higher fungi except for the paper by Denny (**1933**). Denny studied perithecial formation in *Neurospora sitophila* Shear & Dodge. He obtained perithecial production in an atmosphere containing as little as 1.8% oxygen, but not in 0.6% or 0.2% oxygen, or in nitrogen.

The first object of our study was to examine the mucoraceous fungi found in various starters throughout Central Asia to determine if they would grow under anaerobic conditions. Secondly, we wanted to evaluate representative genera from the order Mucorales as to their ability to grow under anaerobic conditions. And, finally, we hoped to determine if lack of oxygen would suppress sexual reproduction by strains of homothallic and heterothallic *Mucor* species.

MATERIALS AND METHODS

Many *Mucor* and *Rhizopus* strains were isolated from numerous samples of Chinese yeast, bubod, ragi, murcha, and lao chao. The samples were collected over a 3-yr period from commercial as well as local markets in Indonesia, India, Taiwan, China, Philippines, and Nepal. Along with representative strains from the ARS Culture Collection, the unidentified strains from these areas were tested for growth under anaerobic as well as aerobic conditions.

All cultures were transferred to potato dextrose agar slants and allowed to incubate 2 wk at 25 C. All representative strains were started from lyophil tubes from our collection and incubated accordingly for 1 wk. Duplicate plates of potato dextrose agar were prepared for each strain and labeled aerobic and anaerobic. Five ml of sterile distilled water was added to the stock slants, which then were mixed vigorously for 5 seconds using a Vortex-Genie®. Using a sterile, disposable 1 ml pipet, we carried out a 3-point inoculum per plate (2 drops per point). The

plates labeled anaerobic were inverted and placed in a GasPak (BBL Microbiological Systems, Cockeysville, Maryland) jar with a disposable hydrogen and carbon dioxide generator and an indicator. Both aerobic and anaerobic plates were incubated for 1 wk at a constant temperature of 25 C. Strains of molds such as *Aspergillus niger* which do not grow in an anaerobic jar were used as controls. After incubation, colonies on the aerobic and anaerobic plates were examined; the diameters of 3 colonies were measured, and the average was determined to the nearest mm. In addition, the appearance of mycelia, yeast cells, and sporangia was noted under a stereoscopic microscope.

Representative strains of *Mucor* from various sources were tested at the Centers for Disease Control (CDC), Atlanta, Georgia, for their ability to grow in eight different atmospheres (air; a candle extinction jar; 15% O_2, 5% CO_2, 80% N_2; 15% O_2, 85% N_2; 5% O_2, 10% CO_2, 85% N_2; 5% O_2, 95% N_2; 5% CO_2, 10% H_2, 85% N_2; and 10% H_2, 90% N_2) on blood agar in plates as described by Lombard *et al.* (**1983**) for testing the relationship of bacteria to oxygen. These authors placed the organisms they tested into six groups on the basis of their ability to grow on the surface of CDC anaerobe blood agar (Dowell *et al.*, **1977**) in four atmospheres (air; candle extinction jar; 5% O_2, 10% CO_2, 85% N_2; and 5% CO_2, 10% H_2, 85% N_2 in an anaerobic system). The groups were defined as follows. (1) Obligate anaerobe: an organism that grows in an anaerobic system but does not grow in 5% O_2, 10% CO_2, 85% N_2; a candle extinction jar; or in air. (2) Microaerotolerant anaerobe: an organism that grows in an anaerobic system and in 5% O_2, 10% CO_2, 85% N_2, but does not grow in a candle extinction jar or in air. (3) Aerotolerant anaerobe: an organism that shows best growth in an anaerobic system; moderate growth in 5% O_2, 10% CO_2, 85% N_2, and less growth in a candle extinction jar and in air. (4) Facultative anaerobe: an organism that shows approximately the same degree of growth in an anaerobic system; 5% CO_2, 10% H_2, 85% N_2; a candle extinction jar, and in air. (5) Microaerophilic aerobe: an organism that shows best growth in 5% O_2, 10% CO_2, 85% N_2; less or no growth in a candle extinction jar, and usually no growth in air or in an anaerobic system. (6) Obligate aerobe: an organism that shows best growth in air and in a candle extinction jar; usually somewhat less growth in 5% O_2, 10% CO_2, 85% N_2, and little if any growth in an anaerobic system.

The sexual reproduction studies were made using mating types that produced zygospores in great abundance on potato dextrose agar under aerobic conditions; duplicate matings were placed in anaerobic jars. The anaerobic matings were made by placing small pieces of mycelia of the two mating types in contact with each other or by placing spores almost in contact with each other.

RESULTS

Of the 60 isolates of *Rhizopus, Mucor,* and *Amylomyces* from ragi, murcha, bubod, Chinese yeast, and similar fermentation starters, all grew under anaerobic conditions in a GasPak jar except for one isolate of *Rhizopus* from murcha from Nepal (TABLE I). On the other hand, several fungi, including *Subbaromyces splendens* Hesseltine, *Aspergillus niger* V. Tiegh., and *Mucor mucedo,* failed to grow under the same anaerobic conditions. These cultures, when transferred back to aerobic conditions, grew normally. *Subbaromyces splendens* might be expected to grow anaerobically since it has been found growing in plugged trickling filter beds, where oxygen levels must be low or nonexistent.

TABLE I shows the diameter of the colonies of various organisms grown in normal ambient air and under anaerobic conditions. It should be noted that neither *Mucor racemosus* nor *M. mucedo* are found associated with starters. *Mucor mu-*

TABLE I

ANAEROBIC GROWTH OF SOME FUNGI, ESPECIALLY THOSE FROM MIXED STARTERS OF THE ORIENT

Strain NRRL No.	Species	Source of culture	Aerobic colony diam at 7 da[a] (cm)	Anaerobic colony diam at 7 da (cm)
5866	*Amylomyces rouxii*	Look Pang, Thailand	7[a]	4
3640	*Mucor racemosus* (−)		7[a]	3
3634	*M. mucedo* (−)	CBS 109.16	5.8[a]	0
337	*Aspergillus niger*	Neuberg 1923	3.7[a]	0
2340	*Subbaromyces splendens*	Trickling filter	7	0
1421	*Mucor javanicus*	CBS 1928 from Kral collection	6.5[a]	1.8
1429	*M. rouxianus*	From CBS 1928	7[a]	1.8
1430	*M. rouxianus*	Blakeslee collection from Klebs	6[a]	3.5
3358	*M. alternans*	Sewage	7	3
3614 (+)	*M. circinelloides*	CBS 192.68	7[a]	3
3615 (−)	*M. circinelloides*	CBS 394.68	7	3
5257	*M. prainii*	Cooke 4752	7	3
13035	*M. javanicus*	Yeast cake IFO 4570	7[a]	4
A25972	*Rhizopus* sp.	Murcha, M6, Nepal	7	3
A25973	*Rhizopus* sp.	Murcha, M7, Nepal	7	6
A25974	*Rhizopus* sp.	Murcha, M7, Nepal	7	0
A25975	*Rhizopus* sp.	Murcha, M3, Nepal	7[a]	7
A25976	*Rhizopus* sp.	Murcha, M8, Nepal	7	5.5
A25527	*Amylomyces rouxii*	Ragi, Java	6[a]	5.8
A21250	*Mucor rouxianus*	Murcha, from L. R. Batra	6.5[a]	4
A25949	*M. circinelloides*	Murcha, Nepal	7	3
A25952	*Mucor* sp.	Murcha, Nepal	7[a]	2.8
A25968	*Mucor* sp.	Murcha, Nepal	7[a]	2.8
A25970	*Mucor* sp.	Murcha, Nepal	7	4
A25971	*Mucor* sp.	Murcha, Nepal	7[a]	3
A25782	*Mucor* sp.	Ragi, Bali	6.8[a]	5.5
A25530	*M. circinelloides*	Ragi, West Java	6.2[a]	3
A25398	*M. circinelloides*	Ragi, Bogor, Indonesia	7	2
A25891	*M. circinelloides*	Ragi, Taiwan, W. Chan	7	3
A25462	*M. circinelloides*	Ragi, Java	7[a]	3.3
A25464	*M. circinelloides*	Ragi, Java	7	3
A25894	*M. indicus*	Ragi, Taiwan, W. Chan	7[a]	4
A25897	*M. indicus*	Sian, China, Lao-Chao	7[a]	3
A25898	*M. indicus*	Sian, China, Lao-Chao	7[a]	3.5

[a] Read at 3 da.

cedo, as a matter of fact, is found growing typically on dung. Schipper (**1976**) considers *M. alternans* V. Tiegh., *M. javanicus* Wehmer, and *M. prainii* Chodat et Nechitch to be synonyms of *M. circinelloides* V. Tiegh. *Mucor rouxii* (syn. *M. rouxianus* Wehmer) was originally isolated from Chinese yeast. However, all of the *Mucor* strains in TABLE I belong in the section *Racemosus* of the genus *Mucor.* Species in this section contain large numbers of chlamydospores in the aerial mycelium (Hesseltine, **1954**).

Each of the strains was selected from different starter cultures for evaluation. In nearly all cases, the diameter of the colonies grown under anaerobic conditions was less than that of the same strain grown under aerobic conditions. The *Mucor* strains grown under anaerobic conditions produced mycelium and also yeast-like cells in abundance; they failed to produce normal sporangia, although sporangiophore initials were often abundant. Some *Mucor* species such as *M. subtilissimus* Oud. also produce abundant yeast-like cells under aerobic conditions. *Mucor*

TABLE II

ANAEROBIC GROWTH OF REPRESENTATIVE SPECIES OF THE ZYGOMYCETES[a]

Strain number	Species	Diam of colonies: Aerobic growth, cm	Diam of colonies: Anaerobic growth, cm	Comments
	MUCORALES MUCORACEAE			
2402 (+)	*Pirella circinans* Bain.	7	0	
1308	*Gongronella butleri* (Lendner) Peyronel et DaVesco	6.5	0	
25507	*Absidia corymbifera* (Cohn) Sacc. et Trotter	4	0	
1365 (+)	*Circinella umbellata* V. Tiegh. et Le Monn.	6.5	0	
2660	*Zygorhynchus moelleri* Vuill.	7	0	
1554 (+)	*Phycomyces blakesleeanus* Burgeff	7	0	
6288	*Syzygites megalocarpus* Ehrenb. ex Fries	7	0	
1459 (−)	*Parasitella simplex* Bain.	7	0	
3104	*Actinomucor elegans* (Eidem) C. Benj. et Hesseltine	7	6.5	Mycelium and yeast-like cells
3469	*Mucor pusillus*[b] Lindt	7	0	
2870	*Rhizopus chinensis* Saito	7	6.5	Mycelium and yeast-like cells
2710	*Rhizopus oligosporus* Saito	7	0	
5866	*Amylomyces rouxii* Calmette	7	4	Mycelium sparse
	THAMNIDIACEAE			
2467	*Thamnidium elegans* Link ex Fries	7	0	
1399 (−)	*Helicostylum piriforme* Bain.	7	0	
2243	*Cokeromyces recurvatus* Poitras	2	2	Yeast-like growth
	RADIOMYCETACEAE			
2753	*Radiomyces spectabilis* Embree	7	0	
3301	*Hesseltinella vesiculosa* Upadhyay	6.5	0	
	MORTIERELLACEAE			
1757	*Mortierella isabellina* Oud.	2.5	0	
2941 (−)	*M. parvispora* Linnemann	6.5	0	
2493	*Haplosporangium bisporale* Thax.	3.8	0	
	CUNNINGHAMELLACEAE			
1388 (+)	*Cunninghamella elegans* Lendner	7	0	
684	*Mycotypha microspora* Fenner	4	2.5	Yeast-like growth
	PILOBOLACEAE			
2289	*Pilaira anomala* (Cesati) Schrot.	7	0	
3168	*Utharomyces epallocaulus* Boedijn	Sparse	0	
	SAKSENAEACEAE			
2443	*Saksenaea vasiformis* Saksena	7	0	
	CHOANEPHORACEAE			
1546	*Gilbertella persicaria* (Eddy) Hesseltine	7	4	Short germ tubes only
2895 (+)	*Blakeslea trispora* Thax.	7	0	
	SYNCEPHALASTRACEAE			
2495 (−)	*Syncephalastrum racemosum* Cohn ex Schroter	7	0	

TABLE II
CONTINUED

Strain number	Species	Diam of colonies: Aerobic growth, cm	Diam of colonies: Anaerobic growth, cm	Comments
	ZOOPAGALES			
	PIPTOCEPHALIDACEAE			
1535	*Piptocephalis repens* V. Tiegh. et Le Monn.	6.5	0	
	KICKXELLALES			
	KICKXELLACEAE			
2642	*Martensiomyces pterosporus* Meyer	4	0	
2237	*Linderina pennispora* Raper et Fennell	6.5	1.5	Mycelium
	DIGMARGARITALES			
	DIGMARGARITACEAE			
2803	*Dispira cornuta* V. Tiegh.	3	1.5	Yeast budding

[a] Growth after 7 da at 25 C. The average is based on the diam in cm of three colonies growing on potato dextrose agar.
[b] Grown at 37 C for 7 da.

genevensis Lendner strains NRRL 1756, 1755, and 1821 were examined and each grew anaerobically, producing yeast-like growth.

Since growth under anaerobic conditions was exhibited by cultures isolated from Asian starters, we decided to investigate whether strains of other Zygomycetes could grow anaerobically. TABLE II shows the results of these experiments using strains of representative genera of 12 of the 14 families recognized in this order (Helicocephalidaceae and Endogonaceae excepted). The results indicate that only species in a few genera will grow under anaerobic conditions. Of the 13 representatives of the family Mucoraceae tested, only *Amylomyces rouxii* grew under anaerobic conditions. Like *Amylomyces, Actinomucor elegans* is used in fermented foods. *Rhizopus chinensis* is related to species found in ragi. *Zygorhynchus moelleri* Vuill. and *Mortierella isabellina* Oud. are typical soil forms that we thought might grow under anaerobic conditions, but they did not. The few Mucorales in other families that did show some indication of anaerobic growth, such as *Cokeromyces, Gilbertella,* and *Mycotypha,* were rather surprising, because nothing in their habitats suggested that they would grow under anaerobic conditions. For example, *Gilbertella* spores germinated and produced short germ tubes, whereas *Cokeromyces* showed swollen yeast-like cells that probably indicated germination of sporangiospores. We found that possibly only 8 of the 31 genera (33 species) tested showed any growth under anaerobic conditions. In addition, we studied five other strains of *Amylomyces rouxii* Calmette (NRRL 2928, 3160, 5192, 5191, 3139). All produced only filamentous growth on the plates, but the amount of growth was variable. Only NRRL 3139 produced abundant aerial growth, and none of the cultures produced any sporangia. Like *Rhizopus,* they failed to produce any yeast cells. This supports our idea that *Amylomyces* was derived in the past from *Rhizopus.*

There was a question about whether five strains representing five genera of the Zygomycetes would grow under anaerobic conditions, so they were grown at

CDC under a variety of aerobic and anaerobic conditions. The results are shown in TABLE III. As the table indicates, none was an anaerobe. The same strains also were grown on potato dextrose agar with the same results.

To further study the growth of *Mucor* under different gaseous atmospheres, we selected six cultures; these were grown on CDC anaerobe blood agar for 48 h at 35 C. The results of this study are shown in TABLE IV. These *Mucor* strains came from five different countries and from four different cultural regions. It should be noted that in an atmosphere of 5% CO_2, 10% H_2, and 85% N_2 under anaerobic conditions, growth was rated just as good as that of the cultures grown in air. However, when CO_2 was omitted (10% H_2, 90% N_2) in an anaerobic environment, the growth of all six strains was less than in the other environment. On the basis of these results, *Mucor* strains can be considered facultative anaerobes.

Mucor species have not been mated under anaerobic conditions. The following cultures were contrasted under appropriate conditions (potato dextrose agar at 25 C for 7 da): *Mucor circinelloides* NRRL 3614 × 3615, *Mucor hiemalis* NRRL 3623 × 3624, *Mucor racemosus* 3640 × 3641, *Mucor lusitanicus* Bruderlin NRRL 3629 × 3631, and *Mucor indicus* Lendner 13133 × 13132. *Mucor lusitanicus* is considered to be a variety of *M. circinelloides* (Schipper, **1976**). When these were mated aerobically, abundant zygospores were present except between NRRL 13133 × 13132. *Mucor genevensis,* a homothallic species, strains NRRL 1755, 1756, and 1821, likewise produced abundant zygospores under aerobic conditions. In no instance when these cultures were mated under anaerobic conditions did any zygospores form in the heterothallic species, as was also true for the homothallic *M. genevensis.* In one plate, a small yellow line formed between NRRL 13133 × 13132. The colonies were typical in growth under anaerobic conditions with mycelium and yeast cells and, in some, sporangiophores present were covered with droplets of exudate but were not terminated by sporangia.

Three strains of *Amylomyces rouxii* (NRRL 2928, 3160, 3139) were studied to determine if anaerobic conditions would alter chlamydospore production. Potato dextrose agar in plates was three-point inoculated and incubated in anaerobic jars at 25 C for 7 da. Chlamydospores were produced in great abundance throughout the aerial mycelium as well as in the substrate mycelium (Ellis *et al.,* **1976**). Since only abortive sporangia were produced, the fungus must reproduce mainly by the enormous number of chlamydospores. In all three strains that had grown anaerobically, chlamydospores were absent except in NRRL 3160. These strains had grown over all or almost all of the agar. We examined a number of microscopic fields and found one or two chlamydospores in NRRL 3160. As far as we know, this is the first report of the suppression of chlamydospore development by anaerobic conditions.

DISCUSSION

The fermentation starters used in China, Indonesia, Nepal, Philippines, Taiwan, and adjacent countries are used to start various food and alcoholic fermentations based upon rice or cassava (Hesseltine, **1983**). The starters are often prepared in small cottage industries and are prepared by making small hard cakes or balls 2–3 cm in diam which contain yeasts, lactic bacteria, and mucoraceous fungi of the genera *Mucor, Rhizopus,* and *Amylomyces.* Even with this restriction of genera of fungi, the species present are limited to just a few in both *Rhizopus* and *Mucor.* One of the characteristics of the *Mucor* species is the fact they all occur in one section of *Mucor* where chlamydospores are found in abundance. Other mucors such as *M. mucedo* and *M. pusillus* in other sections of the genus

TABLE III

DEGREE OF GROWTH AND AERIAL MYCELIUM PRODUCTION ON CDC ANAEROBE BLOOD AGAR IN VARIOUS ATMOSPHERES, GROUPED IN RELATION TO OXYGEN, AND EFFECT OF ADDED CARBON DIOXIDE ON GROWTH OF VARIOUS MOLDS

NRRL number	Microorganism	Degree of growth and aerial mycelium production in atmosphere of:																		Group in relation to oxygen[c]	Effect of added carbon dioxide on growth[d]
		Air		20% O_2 5% CO_2 75% N_2		Candle jar		15% O_2 85% CO_2		15% O_2 5% CO_2 80% N_2		5% O_2 95% N_2		5% O_2 10% CO_2 85% N_2		10% H_2 90% N_2 (anaerobic)		10% H_2 5% CO_2 85% N_2 (anaerobic)			
		GR[a]	AM[b]	GR	AM	GR	AM	GR	AM	GR	AM	GR	AM	GR	AM	GR	AM	GR	AM		
684	*Mycotypha microspora*	2	+	2	+	2	+	2	+	2	+	2	+	2	−	0	−	0	−	OA	LN
2237	*Linderina pennispora*	2	(+)	2	(+)	1	(+)	2	(+)	2	(+)	2	−	2	−	1	−	1	−	OA	LN
2243	*Cokeromyces recurvatus*	3	+	3	+	3	+	3	(+)	3	(+)	2	(+)	2	(+)	0	−	0	−	OA	LN
2870	*Rhizopus chinesis*	3	+	3	+	3	+	3	+	3	+	2	+	2	+	1	−	1	−	OA	LN
3104	*Actinomucor elegans*	3	+	3	+	3	+	3	+	3	+	3	+	3	+	1	−	0	−	OA	LN

[a] Degree of growth: GR = degree of growth, 3 = abundant, 2 = moderate, 1 = sparse, 0 = no growth.
[b] Aerial mycelium: AM = aerial mycelium, + = present, ( ) = poorly developed, − = absent.
[c] Relation to oxygen: OA = obligate aerobe.
[d] Effect of added carbon dioxide: LN = little or no effect on growth.

TABLE IV

RELATIVE GROWTH OF *Mucor* STRAINS ON CDC ANAEROBE BLOOD AGAR[a] AFTER 48 H OF INCUBATION AT 35 C

Mucor strain	*Mucor* species	Source	Ambient air	Candle extinction jar	15% N_2 5% CO_2 80% N_2	15% O_2 85% N_2	5% O_2 10% CO_2 85% N_2	5% O_2 95% N_2	5% CO_2 10% H_2 85% N_2[b]	10% H_2 90% N_2[b]
A25948	*M. circinelloides*	Murcha, Nepal	3+	1+	3+	3+	3+	2+	3+	1+
A23770	*M. indicus*	Look Pang, Thailand	4+	4+	4+	4+	4+	4+	4+	1+
A25462	*M. circinelloides*	Ragi, Java	4+	4+	4+	4+	3+	4+	4+	1+
A25892	*M. circinelloides*	Lao-chao, Taiwan	4+	1+	1+	2+	1+	1+	4+	1+
A25897	*M. indicus*	Lao-chao, Sian, China	4+	4+	4+	4+	4+	4+	4+	2+
A25855	*M. indicus*	Murcha, Nepal	4+	4+	4+	4+	4+	4+	4+	1+

[a] One half plate of anaerobe blood per strain for each atmosphere.

[b] Anaerobic system with palladium catalyst.

are never found in starters and were negative for anaerobic growth. The starter preparations are often made under very poor sanitary conditions, and the question is how can the starters made from previous starters continue to be effective fermentation inocula and still contain the same few kinds of microorganisms? The starters, besides the microorganisms, are made from rice flour and other ingredients, including certain spices. After inoculation with starter, the moist cakes are incubated at 25–30 C on muslin-covered bamboo trays and gradually air-dried for 2–5 da. Finished cakes often are extremely hard and compact, grayish white, with a dusty surface. When the cakes are dry they are ready for sale, either in packages or unpackaged. Hesseltine (**1983**) has speculated that the spices, which are known to be inhibitory to many bacteria and molds, are the agents that select the right population of microorganisms for fermentation. From this study it is apparent that development of anaerobic conditions within the starter balls may also be selective, since all the isolates of *Mucor, Amylomyces,* and *Rhizopus* grew well under anaerobic conditions, especially when CO_2 was supplied. Certainly the bacteria and yeasts, also found in the starter cakes, could produce the CO_2 required by the starter molds. Thus, at least three factors can be selective for the development of the starter molds: (1) the selective action of spices, (2) anaerobic conditions, and (3) the presence of CO_2.

From the data in TABLE II it is apparent that facultative anaerobes are not common, even in the Mucorales.

We have mating types of some of the species that grow under anaerobic conditions, so we decided to see if they would produce zygospores under anaerobic conditions. Mating pairs were selected that have strong mating reactions when contrasted on potato dextrose agar under aerobic conditions. However, under anaerobic conditions no zygospores were formed and no yellow line between colonies, which is an indication of an early sexual stage, was formed. Denny (**1933**) investigated perithecium formation in *Neurospora* but found that at O_2 levels of less than 0.8%, no perithecia were formed.

Emerson and Weston (**1967**) discovered and described a new species in the Leptomitales, *Aqualinderella fermentans,* which thrives in the absence of oxygen and degrades glucose solely by a homolactic fermentation. This fungus produces oogonia in culture, presumably under anaerobic conditions, provided CO_2 is present. This is perhaps the best documented case of anaerobic development of the sexual state in lower fungi. Our studies indicate that zygospores are not produced under anaerobic conditions in the Zygomycetes.

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